secondary to potentiated sarcoplasmic reticulum Ca release and enhanced Na/Ca exchange (NCX), and can be abolished by the I_{Na} blocker tetrodotoxin, but not by the selective (in ventricles) late I_{Na} blocker ranolazine.
Aim. Since repolarization in human atrial myocytes is relatively rapid and potently modulated by Ca (as in mouse ventricle), we investigate whether the same EAD mechanism may occur in human atria. Indeed, phase-3 EADs have been suggested to underlie re-initiation of atrial fibrillation (AF) after termination upon autonomic stimuli - well recognized AF triggers.
Methods: We integrated a Markov model of I_{Na} (also describing the channel interaction with ranolazine, similar to work by Clancy) in our human atrial myocyte model. To recapitulate experimental results, we simulated rapid cell pacing (10 Hz) in the presence of Acetylcholine (0.1 μM) and Isoproterenol (1 μM), and assessed EAD occurrence upon return to sinus rhythm (1 Hz).
Results: Cellular Ca overload during fast pacing results in a transient period of hyper-contractility after return to sinus rhythm. Here, fast repolarization and enhanced NCX facilitate I_{Na} reactivation via the canonical gating mode (i.e., not late I_{Na} burst mode), which drives EAD initiation. Notably, in action potential clamp experiments, an EAD waveform elicits a lidocaine-sensitive inward current during the EAD upstroke in pig atrial cells. Simulating ranolazine administration reduces peak I_{Na} and leads to a faster repolarization, during which I_{Na} fails to reactivate.
Conclusions: Our simulations suggest that non-equilibrium I_{Na} reactivation critically contributes to arrhythmias in human atrial myocytes. Ranolazine might be beneficial in this context by blocking peak (not late) atrial I_{Na}.

982-Plat
Probing the Trafficking Routes of KCNQ1 and KCNE1 After Their ER Exit
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Background: KCNQ1 (Q1, pore-forming channel subunit) and KCNE1 (E1, regulatory subunit) associate to form the slow delayed rectifier (I_{Ks}) channel, important for cardiac action potential repolarization. Although Q1/E1 are obligatory partners in cardiomyocytes, in adult ventricular myocytes the two are not well colocalized: E1 is on the lateral cell surface while Q1 is mainly in the intracellular junctional SR. This appears to contradict data from heterologous expression or non-cardiac cell types, which suggest that Q1/E1 assemble into I_{Ks} when they are still in ER or soon thereafter. The current study is designed to distinguish between 2 scenarios. First, Q1 and E1 traffic from ER to the plasma membrane together, followed by Q1 internalization to the ER/SR compartment. Alternatively, Q1 and E1 traffic on different routes from ER to their separate destinations.
Methods: COS-7 cells transfected with Q-GFP/E1-dsR are cultured in presence of brefeldin A (BFA) for 12 – 16 hr, to allow protein translation without ER exit. Cells are imaged in absence of BFA but presence of cycloheximide+dynasore (blocking protein translation and endocytosis). Time-lapse images (Zeiss 710, 37°C) of 4-um optic slice (to maximize capture of trafficking events in thin cytosol of COS-7 cells) are recorded till the Q1-GFP and E1-dsR distribution reaches quasi steady-state (> 2 hr).
Results: BFA removal allows ER exit and Golgi reconstruction. E1-dsR exits ER in distinct vesicles, more frequently at the cell periphery. These vesicles gradually cluster to the peri-nuclear region, entering the post-BFA Golgi. Q1-QFP stays in the ER compartment for > 2 hr. Its ER location is confirmed by FRAP experiments.
Conclusion: Our data support the second scenario. Experiments are in progress to test whether E1-dsR and Q1-GFP behave the same when expressed in a cardiac myocyte environment.

983-Plat
Trafficking and Gating Mechanisms of HERG1A C-Terminus (LQTS-2) Truncation Mutations on HERG1A-HERG1B Hetero-Multimeric Channel
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In heart, HERG1A-HERG1B subunits generate the rapid component of delayed rectifier potassium current, IKr, critical for normal repolarization. Congenital mutations in HERG1A lead to long QT syndrome-2 (LQTS-2), a condition that increases susceptibility to fatal arrhythmias. Many LQTS-2 mutations that are localized to HERG1A C-terminus generate truncated variants and their behavior when co-expressed with HERG1B and their (patho) physiological effects in heart are largely unknown. Therefore, we compared the physiological impact and mechanistic bases of two HERG1A C-terminus truncation mutations (G965X, R1014X). Bungarotoxin-binding optical assays for channel cell-surface expression and electrophysiological recordings were carried out in Human Embryonic Kidney cells. R1014X displayed normal surface density whereas, the G965X mutant, displayed a significant decrease in channel surface density, which was fully rescued by wild-type HERG1A-HERG1B subunits. Homo-multimeric (mutant+HERG1B), G965X and R1014X channel subunits yielded currents with severely reduced current amplitude. When co-expressed with wild-type HERG1A, both mutants exerted a dominant negative effect but to different extents: G965X current amplitude was partially rescued while R1014X current remained unchanged. Homo-multimeric mutant channels displayed a significant rightward shift in the activation curve which was partially (G965X), or not (R1014X) rescued with wild-type HERG1A. The data reveal these mutants exert a purely biophysical effect on hetero-multimeric (mutant HERG1A + wild-type HERG1A + HERG1B) channels. These mechanistic insights may enhance therapies for LQTS-2 mutations. Furthermore, we show for the first time that the R1014X shows normal surface expression, but is non-functional and exerts a strong dominant-negative effect on wild-type HERG1A channels which is in contrast to existing data that demonstrates that R1014X causes HERG1A channel dysfunction by defective trafficking. Finally, studies that explore the functional impact of these mutations in heart will provide novel information that will be more predictive of disease penetrance.

984-Plat
Dominant Negative Consequences of a HERG 1B Mutation Associated with Intrauterine Fetal Death
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The human ether-a-go-go related gene (hERG) encodes the voltage-gated potassium channel responsible for conducting the rapid delayed rectifier potassium current (IKr). Reduced IKr slows cardiac action potential repolarization and is an underlying cause of cardiac arrhythmias associated with both inherited and acquired long QT syndrome (LQTS). Two subunits comprise the hERG channel, hERG 1a and hERG 1b, both of which represent critical components of cardiac repolarization. R25W is a hERG 1b mutation associated with a case of intrauterine fetal death and previously shown to reduce heteromeric hERG 1a/1b current density in Chinese hamster ovarian cells1. The mechanism of current reduction and a direct link to cardiac dysfunction has not been described for R25W. We expressed R25W in HEK cells and human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) to define the molecular, biophysical, and physiological consequences of R25W. R25W significantly reduced 1a and 1b protein levels to 76.4 ± 6.0% and 65.2 ± 7.8% of control, respectively, in HEK cells coexpressing hERG 1a and 1b. In iPSC-CMs, R25W transfection significantly reduced peak tail IKr (0.8 ± 0.1 pA/pF, n = 10, $p < 0.05$) compared to either native IKr (1.7 ± 0.2 pA/pF, n = 5) or wild type 1b transfected iPSC-CMs (1.5 ± 0.3 pA/pF, n = 7). Interestingly, wild-type 1b did not affect peak tail IKr whereas transfection of either 1a alone (35.9 ± 11.0 pA/pF, n = 5) or 1a/1b together (24.8 ± 11.2 pA/pF, n = 5) dramatically increased current density. These data identify R25W as a dominant negative mutation that reduces native IKr and provide new evidence that hERG 1b fails to traffic as a homomeric channel in human cardiomyocytes. Supported by NIH/R01HL081780 and the UW Training Program in Translational Cardiovascular Science (T32HL007936).
1. Crotti et al., 2013, JAMA.

Platform: Membrane Pumps, Transporters, and Exchangers II

985-Plat
On the Na^+/H^+ Selectivity of Membrane Transporters and Enzymes: Experimental and Theoretical Studies of an ATP-Synthase Rotor Ring
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Electrochemical gradients of sodium and protons are the primary driving forces for a wide array of cellular processes mediated by membrane proteins, such as energy conversion, solute uptake and multi-drug extrusion. The factors that confer ion specificity to these systems are poorly understood. Sodium and proton-driven systems of different functionality are often found in the same organism, and membrane proteins within the same functional family frequently feature distinct specificities despite a high similarity in their structures. Therefore, it appears that the specificity of H^+/Na^+-coupled systems is largely